



Study on the Antibacterial and Bactericidal Effects of *Ailanthus altissima* Leaves Extract

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In present studies, the antibacterial and bactericidal effect of the *A. altissima* leaves extract are reported. Three different concentrations of *A. altissima* leaves extract were tested for the activities of antibacterial and bactericidal on *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The inhibition zone diameter of the three bacteria was tested using the method of filter paper diffusion. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were tested by the double dilution method. Three different concentrations of *A. altissima* leaves extract all have antimicrobial effect. Therefore, the inhibitory effect is more evident at higher concentration of the drug. The same concentration of *Ailanthus altissima* leaves extract on different amounts of bacterial suspensions have inhibitory effect, the greater the amount of bacterial suspension, the bigger inhibition zone diameter, the inhibitory effect is more evident. The minimum inhibitory concentrations of *A. altissima* leaf extract against *S. aureus*, *P. aeruginosa*, *E. coli* were 35.0, 8.8 and 70.0 mg/mL, respectively. The inhibitory effect of *P. aeruginosa* is the strongest. The minimum bactericidal concentration of *A. altissima* leaf extract against *S. aureus*, *P. aeruginosa*, *E. coli* were 35.0, 17.5 and 70.0 mg/mL, respectively. Therefore, the bactericidal effect on *P. aeruginosa* is the strongest.

Key Words: *Ailanthus altissima*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Minimum inhibitory concentration, Minimum bactericidal concentration.

INTRODUCTION

The emergence and spread of microbial resistance is growing each day. *Staphylococcus aureus* Rosenbach and *Pseudomonas aeruginosa* are the predominant cause of fatal burn wound sepsis and isolation of multi-drug resistant strains is a common problem in hospitals, leaving minimal or no effective systemic treatment options for the clinician. Increasing resistance of *P. aeruginosa* to many antibiotics has been observed and poses a therapeutic dilemma¹. Thereby it is necessary to develop new antimicrobials of natural or synthetic origin. As far as the natural sources are concerned, apart from the microbial sources, plants appear to be valuable antimicrobial resources. Plants can produce a large number of secondary metabolites that may exceed a hundred thousand molecules². All of these don't have antimicrobial potential, but some of them can produce significant activity against the human pathogens. This activity is not necessarily of the same magnitude as that of the prevailing antimicrobials, but still provides some scope for optimism³. Natural medicinal products have been used for millennia to treat multiple ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently resurgence of interest by physicians in natural products. Consequently, the search for such antimicrobials has intensified during recent times.

Ailanthus altissima (Mill.) Swingle is a deciduous arbor, which is a member of Simaroubaceae family. It is mainly distributed in the southeast Asia. In China, it is mainly distributed in GuangDong, GuangXi, YunNan, the south of LiaoNing Province and many other places. *Ailanthus altissima* leaves contain many active substances such as sterol, triterpene, tetracyclitriterpene, diterpenoid and alkaloid, etc. Different parts of the plant are used in the indigenous system of medicine for the treatment of various human ailments such as wounds, ulcers, bowels and cholera. Pharmacological investigations indicated that its bark, fruit and leaves possess antibacterial, anticancer, antiinflammatory, spasmolytic. In China, *Ailanthus altissima* barks are extensively used to stop diarrhea⁴. According to the record of relative documents, *Ailanthus altissima* leaves have the function of antiinflammation, detoxification, disinfestations of pests. Besides, they can be used to treat enteritis, dysentery, furuncle, carbuncle, rhus dermatitis and tinea tonsure⁵. At present, the study on their application (for instance, pharmacological characters) is very rare. Upon this, the research on the antibacterial and bactericidal effects of *Ailanthus altissima* leaves is conducted so as to provide the theoretical evidence for the reasonable application of *Ailanthus altissima* leaves^{6,7}.

EXPERIMENTAL

Bacterial strain and inoculum standardization:

Staphylococcus aureus, *P. aeruginosa* and *Escherichia coli* procured from Beijing microbial institute; beef extract (Beijing ShuangXuan microbiological enterprise); peptone, yeast leaching powder, Agar (Beijing aoboxing biological technology Co.); sodium chloride, sodium hydroxide (analysis pure) (Beijing chemical reagent company).

Sterile work station: DL-CJ-2V medical type clean bench (Beijing DongLian Harbin equipment manufacturing Co.), rotate evaporator RE-52 A (Shanghai biochemical instrument factory), circulation and multi-purpose vacuum pump SHB-III (zhengzhou (Great Wall Co.), constant temperature oscillator THZ-82 (Changzhou Guohua electric appliance Co.), biochemical incubator LRH-150 (Shanghai A huan scientific instruments Co.), YX280 type stainless steel steam sterilizer (Shanghai three shen medical equipment Co.).

Ailanthus altissima leaves extraction: The *Ailanthus altissima* leaves extract was prepared as follows: In first step, 2.5 kg *Ailanthus altissima* leaves have been dried to constant weight, then grinding them and adding 10 L of distilled water. After 2 h of heating, refluxing and extracting, the water was concentrated into 50 % of the original one. Lastly, the concentrated liquid was reserved with cryopreservation. The high concentration of *Ailanthus altissima* leaves extract could be obtained by concentrating 1 L of the low concentration extract into 50 % of itself. High levels *Ailanthus altissima* leaves extract equivalent concentration is 70 mg/mL. Medium levels *Ailanthus altissima* leaves extract equivalent concentration is 35 mg/mL. Low levels *Ailanthus altissima* leaves extract equivalent concentration is 24.5 mg/mL.

Radial diffusion assay: For detection of antimicrobial activity, a sensitive radial diffusion technique was used as described earlier⁸. Using a solid agar medium, the agar layer consisted of beef extract 3 mg/mL broth system, with 0.01 % peptone and 0.8 % agar phase pH 7. The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to McFarland standards. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Ca. 100 µL of the crude extract were introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37 °C. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The

plates were observed for zones of inhibition after 24 h. The diameter of the clear zone was measured. The effects were compared with streptomycin at a concentration of 1 mg/mL, respectively.

Minimum inhibitory concentration (MIC): The estimation of MIC of the crude extracts was carried out using the two-fold dilution method according to Clinical and Laboratory Standards Institute guidelines⁹. Two-fold dilutions of the crude extracts were prepared and 2 mL different concentrations of the solution were added to 18 mL of pre-sterilized molten nutrient agar. The medium was then poured into sterile petri dishes. The bacterial isolates 0.1 mL were added to the dishes. The plates were later incubated at 37 °C for 24 h for bacteria, respectively, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Minimum bactericidal concentration (MBC): The minimum bactericidal concentration of the plant extracts was determined by a modification of the method of Spencer and Spencer¹⁰. Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar plates and later incubated at 37 °C for 48 h for bacteria. The minimum bactericidal concentration was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

RESULTS AND DISCUSSION

Radial diffusion assay of *Ailanthus altissima* leaves

extract: All three extracts of the plant tested showed varying degree of antibacterial activities against the test bacterial species. Experimental data is shown in Table-1. The antibacterial activities of extracts compared favourably with that of standard antibiotic (streptomycin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The inhibition zone for *Pseudomonas aeruginosa* was much more (10.1-13.5 mm) as compared to other bacteria. The high levels *Ailanthus altissima* leaves extract (inhibition zone 9.9-13.5 mm) was found to be more effective than the low levels *Ailanthus altissima* leaves extract (inhibition zone 8.7- 13.0 mm) against all the organisms.

Minimum inhibitory concentration (MIC) of *Ailanthus altissima* leaves extract: The estimation of minimum inhibitory concentration of the crude extracts was carried out using the two-fold dilution method. Liquid nutrient medium has bacterium,

TABLE-1
ANTIBACTERIAL ACTIVITIES PROFILE OF THREE DIFFERENT CONCENTRATIONS OF *Ailanthus altissima* LEAF EXTRACTS

Test bacteria	Bacterial suspensions (mL)	Zone of inhibition (mm)			
		Ailanthus leaf extracts (24.5 mg/mL)	Ailanthus leaf extracts (35.0 mg/mL)	Ailanthus leaf extracts (70.0 mg/mL)	ST (1 mg/mL)
<i>Escherichia coli</i>	0.1	8.67	9.00	9.85	0
	0.5	10.95	10.32	11.52	0
	1.0	11.11	11.32	12.32	0
<i>Staphylococcus aureus</i>	0.1	9.16	8.96	10.73	13.2
	0.5	10.32	10.25	11.20	14.3
	1.0	11.95	11.24	12.43	14.9
<i>Pseudomonas aeruginosa</i>	0.1	10.65	10.44	10.14	20.0
	0.5	11.23	11.32	11.60	21.6
	1.0	12.98	13.03	13.51	22.3
ST-Streptomycin.					

TABLE-2
MINIMUM INHIBITORY CONCENTRATION OF THE EXTRACTS OF THE *Ailanthus altissima* LEAF

Testbacteria	Concentration(mg/mL)								
	Negative	70.0	35.0	17.5	8.8	4.4	2.2	1.1	Positive
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	+	+	+	+
<i>Escherichia coli</i>	-	-	+	+	+	+	+	+	+
Distilled water	-	+	+	+	+	+	+	+	+

+: Presence growth of the test microorganism. -: Absence growth of the test microorganism.

but no medicine as the positive; liquid nutrient medium has medicine, but sterile as the negative (Table-2). The minimum bactericidal concentration (MIC) of *A. altissima* leaves extract against *S. aureus*, *P. aeruginosa*, *E. coli* were 35.0, 8.8 and 70.0 mg/mL, respectively. The inhibitory effect on *P. aeruginosa* is the strongest.

Minimum bactericidal concentration (MBC) of *Ailanthus altissima* leaves extract: The minimum bactericidal activity of the extract for different bacteria ranged between 17.5 and 70.0 mg/mL. The minimum bactericidal concentration of *A. altissima* leaf extract against *S. aureus*, *P. aeruginosa*, *E. coli* were 35.0, 17.5 and 70.0 mg/mL, respectively. Therefore, the bactericidal effect on *P. aeruginosa* is the strongest. It may be attributed to the presence of soluble sterol and alkaloid compounds. The inhibitory effect of the extract of *Ailanthus altissima* leaves against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens.

Conclusion

Three different concentrations of *Ailanthus altissima* leaves extract have antimicrobial effect. The same concentration of *Ailanthus altissima* leaves extract on different amounts of bacterial suspensions have inhibitory effect, the greater the amount of bacterial suspension, the bigger inhibition zone

diameter, the inhibitory effect is more evident. The minimum inhibitory concentrations of *Ailanthus altissima* leaves extract against *S. aureus*, *P. aeruginosa*, *E. coli*, the inhibitory effect of *P. aeruginosa* is the strongest. The minimum bactericidal concentration of *A. altissima* leaf extract against *S. aureus*, *P. aeruginosa*, *E. coli* were 35.0, 17.5 and 70.0 mg/mL, respectively, the bactericidal effect on *P. aeruginosa* is the strongest. So *Ailanthus altissima* leaves extract is a potent anti *P. aeruginosa* and anti *S. aureus* drug.

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